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# The RNA Interference System Differently Responds to the Same Mobile Element in Distant *Drosophila* Species

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Mobile genetic elements (MEs) account for a considerable portion of the genome in almost all organisms studied in this respect [1,2]. During evolution, organisms acquired numerous protective mechanisms that control the activity of various MEs and prevent their transpositions [3,4].

In this work, we analyzed molecularly the RNA interference-related response of the host genome to a new foreign ME. We characterized a class of short RNAs that were found in *Drosophila melanogaster* cells after the introduction of the *Penelope* retroelement isolated from the *Drosophila virilis* genome.

Hybrid dysgenesis provides a brilliant example of ME mobilization, which causes various mutations and other genetic alterations, including gonadal sterility [5,6]. Three independent systems of hybrid dysgenesis have been described to date in *D. melanogaster*, and the above alterations result in each case from activation of one ME: *P* element, *I* element, or *hobo* [5–7]. On the other hand, at least six unrelated MEs are mobilized in hybrid dysgenesis in *D. virilis* [8,9]. A key role in their mobilization is ascribed to *Penelope* [10,11].

In both *D. melanogaster* and *D. virilis*, hybrid dysgenesis always occurs in the progeny resulting from crosses of only one direction, namely, when a male whose genome contains active copies of the transposon responsible for dysgenesis is crossed with a female whose genome lacks full-length active copies of this ME [6,11]. However, recent studies have shown that, apart from active ME copies, the *D. melanogaster* genome contains certain loci, such as *flamenco* and *42AB*, that harbor fragments or divergent copies of various ME. Short RNAs synthesized from such loci provide a kind of immunity against ME reinvasion and mobilization, thus preventing their adverse effects [12,13].

We have previously constructed transgenic *D. melanogaster* strains by introducing the full-length copies of *Penelope* cloned from the *D. virilis* genome via *P*- mediated transformation. An analysis showed that *Penelope* was amplified and experienced multiple transpositions in the new host genome [14]. A special study revealed that *Penelope* is completely absent from the genome of *D. melanogaster*, which belongs to another subspecies and is separated from *D. virilis* by 50 Myr of divergent evolution. Thus, we had a unique opportunity to study the

behavior of a ME experimentally introduced in the genome of *D. melanogaster* that has never been invaded by this ME before.

Examination of various *D. virilis* strains revealed two classes of short RNAs (siRNA, 20–22 nt and piRNA, 23–29 nt) that were homologous to *Penelope* (Fig. 1). It is possible that generation of these RNAs and a balance between the two classes in germline and somatic cells controls *Penelope* transpositions in dysgenic crosses (unpublished data).

It is of interest that only one class (20–22 nt) of short RNAs homologous to *Penelope* was found in transgenic *D. melanogaster* strains. We did not detect piRNAs (23–29 nt), while RNAs of this class are observed for other endogenous MEs present in this species (Fig. 1).

This finding was independently verified in special experiments using mutations of the genes that control various steps of piRNA biogenesis. The experiments showed that mutations of the *armi* and *spindle-E* genes did not increase the level of *Penelope* expression in the transgenic *D. melanogaster* strains (Fig. 2).

Several interesting findings were made when studying the distribution of siRNA (20–22 nt) peaks along the *Penelope* sequence. For instance, in the transgenic *D. melanogaster* strains, the peaks often coincided with the *Penelope* long terminal repeats, which are frequently inverted (Fig. 3). It is possible that double- stranded hairpins formed in the transcripts of such copies serve as a source of 20- to 22-nt RNAs. Note that RNAs of this class are uniformly distributed along the *Penelope* sequence in *D. virilus* strains, and their biogenesis is possibly related to transcription of both strands (Fig. 3).

Our results indicate that the RNA interference system may differently respond to the appearance of active ME copies in the genome. When a ME has already invaded the genome of a species, its cells have piRNAs that are homologous to the ME and interact with its transcripts to induce their degradation. This seems to be the case when crosses are performed between *D. virilis* strains that have different active MEs of the *Penelope* family. On the other hand, when *Penelope* was introduced via transformation into the genome of the distant species *D. melanogaster*, only siRNAs (20–22 nt) were produced, and these siRNAs seemed to be incapable of completely inhibiting transcription and transposition of *Penelope* in the new host genome.

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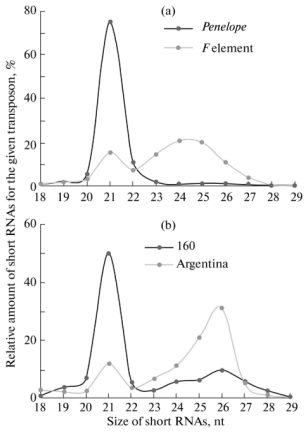


**Fig. 1.**

Short interfering RNAs isolated from the ovaries of (a) a transgenic *D. melanogaster* strain (strain 27) transformed with *Penelope* and (b) *D. virilis* strains (160 and Argentina).

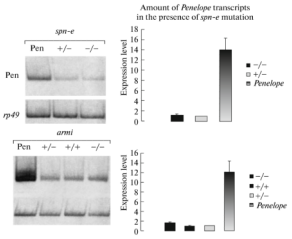


**Fig. 2.**

Effect of mutations affecting the piRNA-dependent pathway on the *Penelope* transcription level estimated by RT–PCR.

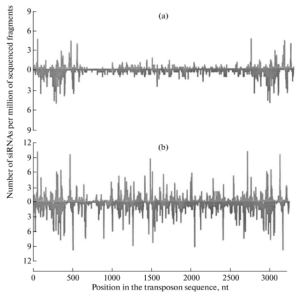


**Fig. 3.**
Distribution of short RNAs (20–22 nt) along the *Penelope* sequence in (a) transformed *D. melanogaster* strain (XXC) and (b) *D. virilis* strain 160.